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BLACK LIGHT MEDIATED GROWTH AND SPORULATION OF *MAGNAPORTHE ORYZAE*

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ABSTRACT

Blast fungus *Magnaporthe oryzae* (T.T. Hebert) M.E. Barr (Ana: *Pyricularia grisea*) is one of the important fungal pathogen which affects rice cultivation by causing rice blast disease. Rice blast is one of the major diseases causing recurring yield losses in all the rice growing regions of the world. An attempt is made to identify optimum nutrient medium and the nature and duration of light requirements for growth and sporulation. Monosporic culture of the fungal pathogen was grown on different media like Corn Meal Agar (CMA), Oat Meal Agar (OMA), Rice Straw Agar (RSA), Potato Dextrose Agar (PDA), V8 juice Agar (V8A) and growth rate of the culture was recorded up to 11 days, amount of sporulation was compared in different media where cultures were allowed to grow and sporulate under the fluorescent, dark, black light and alternate black light and dark (16:8) conditions. The cultures grown on V8 juice agar and oat meal agar showed significant sporulation of $5.5x10^4$/ml and $4.7x10^4$/ml and radial growth of 85 mm and 90 mm respectively under the continuous exposure to black light.

KEYWORDS: *Magnaporthe oryzae,* Blacklight, Conidia, Sporulation, Blast Fungus, Rice

INTRODUCTION

Rice blast disease caused by ascomycetes filamentous fungus *Magnaporthe oryzae* (T.T. Hebert) M.E. Barr (Ana: *Pyricularia grisea*) is an important disease of rice, causes significant losses in yield (Khush and Jena, 2009). It is one of the major diseases causing recurring yield losses in all the rice growing regions of the world (Valent et al., 1991). Different species of *Magnaporthe* cause blast disease on broad range of monocot plants. This disease is a continuous threat to the rice production in the rice cultivars in all the rice growing regions. Rice blast fungus has the potency to cause disease in all stages of growth of the host rice plant. *M. oryzae* initially grows biotrophically in infected tissues (Kankanala et al., 2007) Eventually, lesions develop on rice plants and the fungus produces more conidia to reinitiate the infection cycle (Ribot et al., 2008).

M. oryzae fungus shows high morphological and genetic variations corresponding to the rice cultivars. *M. oryzae* and rice plant has been considered as a model system in the study of host-fungal pathogen interactions (Dean et al., 2012). Many researchers have characterized the *M. oryzae* pathogen at various levels. Still there is a lot of gap to understand about the basic and molecular mechanism and events occurring during host infection. There is a demand for universal procedure to isolate, culture and maintenance of this rice blast pathogen *M. oryzae*. Higher density spore suspension is required for successful versatile host pathogen studies (Partridge-Metz and Chandra, 2011). Many researchers have reported that growth rate and sporulation is influenced by culture media and physical factors (Guochang and Shuyuan, 2001; Netam et al., 2013; Hayashi et al., 2009). Black light is one of the important factors known to regulate the sporulation in fungi. Information on a universal reliable growth media for sporulation and specific light exposure for maximum

sporulation is not available for this important plant pathogenic fungus. Thus an attempt is made in the present experiment towards achieving this.

Lee et al., (2006) demonstrated that involvement of complex regulation of light for asexual fungal development and role of blue light in spore formation. Blue light has been regarded as an important stimulus for conidial formation. By using the formerly introduced culture media, we could not obtain sufficient conidia. Hence, we tried to find the optimized condition in which *M. oryzae* sporulates best by growing the pathogen in different media and exposing the culture to different sources of light.

MATERIALS AND METHODS

Fungal Source

M. oryzae culture was obtained from Microbial Type Culture Collection (MTCC No. 1477) and also *M. oryzae* was isolated from the diseased rice blast leaf samples collected from V.C. Farm Mandya district, Karnataka, India (Lat: 12°33' Long: 76°49').

Effect of Different Culture Media and Different Qualities of Light on Mycelial Growth and Sporulation of M. oryzae

Monoconidial culture of the pathogen was established using standard procedure from the infected leaf lesions. (Hayashi et al., 2009). Five different culture media *viz.*, Corn Meal rice straw Agar medium (CMA), Rice Straw Agar (RSA), Oatmeal Agar Medium (OMA), Potato Dextrose Agar medium (PDA), and V-8 juice Agar medium (V8A) were used for culturing the pathogen (Guochang and Shuyuan, 2001; Hayashi et al., 2009). All media were sterilized at 121°C for 15 minutes and then each culture medium with streptomycin sulphate at 20mg/lt was poured into 90mm Petri plates. After solidification of the medium in Petri plates, 5mm diameter disc of monoconidial culture of the fungus was placed in the centre of the plates and incubated at 27±1°C exposing them for 24 hrs to different qualities of light vertically from 20 cm height *viz.*, fluorescent light (Philips champion TL/40W), black light (Narva LT-T8 Black light blue, LT 18 W/073, Germany) with the wavelength range of 350-390nm (Figure 1), 16 hr black light and 8hr dark, dark, and natural light (room condition). There were three replicates for each treatment and experiment was repeated thrice. The mycelial growth, pigmentation and colony diameter were recorded every 24 hours for 11 days.

Spore Count

Conidia were counted on the 12th day of incubation. Three discs of 5 mm diameter culture were taken using cork borer from different locations randomly from each Petri dish. Each disc was placed in a test tube containing 1 ml sterilized water and shaken thoroughly for the detachment of the conidia from the mycelia discs. The number of spores per ml was quantified using haemocytometer (Hayashi et al., 2009).

Statistical Analysis

Tukey HSD test - ANOVA (analysis of variance) was used to test the significance of differences in growth and sporulation. This was done to compare five different treatments. All analysis was done using SPSS statistical software.

RESULTS

Effect of Different Culture Media and Different Qualities of Light on Mycelial Growth

Among the five media, mycelia growth was found to be highest in V8 Agar medium under fluorescent light

condition (Table 1) Under continuous exposure to dark, mycelia growth was significantly more in OMA among the five media tested. In natural light conditions (which is taken as control) also OMA supported maximum growth which is followed by CMA, PDA, V8A and RSA showed significantly less growth. When continuous black light was given there was a significant variation in the growth rate on different culture media. OMA and PDA showed maximum growth followed by V8A, CMA and least growth was observed in RSA. In case of alternate 16 hrs black light and 8 hrs dark light exposure treatment there was no significant change in the cultural growth in different media.

Effect of Different Culture Media and Different Qualities of Light on Sporulation

Amount of sporulation varied in different media exposed to different qualities of light (Table 2). There was no sporulation in the presence of fluorescent light in all the media except in OMA where $4.44X10^2$ spores/ml was observed. In the dark condition sporulation was observed only on OMA and PDA. Amount of sporulation was higher in OMA as compared to PDA (figure 2). Under natural condition minimal sporulation was observed only in CMA medium. Under continuous black light blue condition except RSA medium all the remaining four culture media showed sporulation. In CMA, OMA, PDA, V8A the spore count was $4.88X10^3$ spores/ml, $4.71X10^4$ spores/ml, $4.44X10^2$ spores/ml, $5.56X10^4$ spores/ml respectively (figure 2). Under black light blue and dark, CMA induced significantly higher sporulation than other media tested (figure 2) Even though sporulation was observed on all media under black light and dark, the influence of black light was more on inducing higher sporulation on V8A and OMA.

DISCUSSIONS

Present investigations revealed that *M. oryzae* shows variation in the growth rate when grown in different culture media exposed to different qualities of light. Several other workers also showed the effect of different culture media on the growth of blast fungus (Partridge-Metz et al 2011; Netam et al., 2013; Lodhi et al., 2013). However, their reports are limited to the comparison of mycelia growth on different media exposed to light and dark cycles.

Our results on sporulation are in accordance to those of Guochang and Shuyuan, (2001) who observed that maximum sporulation of *M. oryzae* occurred under black light conditions. However, they obtained maximum sporulation in CMA and the present study revealed that the best medium is V8A for the same condition. Present investigation also revealed that OMA is suitable for induction of sporulation under varied light conditions. Similar attempt for sporulation under black light was done by Hau et al. (1980) and they developed the system for sporulation of *Bipolaris oryzae* using blacklight. Recently Hosseini-moghaddam et al., (2013) investigated the effect of photoperiod on sporulation of *P. oryzae* and the sporualtion induction was reported after 20^{th} day. The present investigation revealed that 12days are sufficient for inducing sporulation with continous black light exposure.

Current research work revealed that the conidial production of *M.oryzae* was greatly influenced by the culture media also. V8A and OMA appeared to be the best media for conidial production of *M. oryzae*, and also suitable media for mycelial growth of the fungus. However, PDA was reported as the best medium for vegetative growth and sporulation (Lodhi et al., 2013)

Vegetative mycelia growth was not influenced by light exposure as observed in Table 1 indicating that *M. oryzae* growth is light independent.

The present study showed that ideal culture medium and light with optimum range of wavelength are critical for good mycelia growth and sporulation (conidial production) for *M. oryzae*. This is in agreement with the reports that vegetative growth and sporulation of many fungi are influenced by physical and biological factors (Yi et al., 2008; Hau et al., 1980). All the earlier reports showed the requirement of more than 15 days for sporulation (Hosseini-moghaddam and Soltani, 2013; Lodhi et al., 2013).

CONCLUSIONS

Present study revealed that induction of good amount of sporulation is possible in 12 days itself by exposing the Magnaporthe oryzae cultures to continuous blacklight. The optimum media for sporulation were identified as V-8 agar and Oat meal agar. This method is thus time saving for many investigations involving host pathogen interactions which requires good amount of spores for inoculation.

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APPENDICES

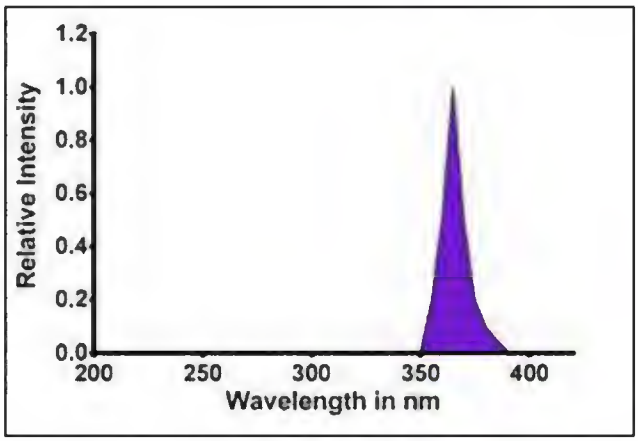
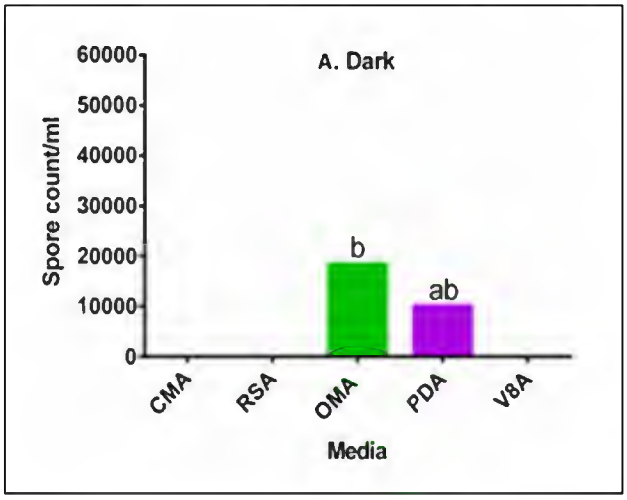
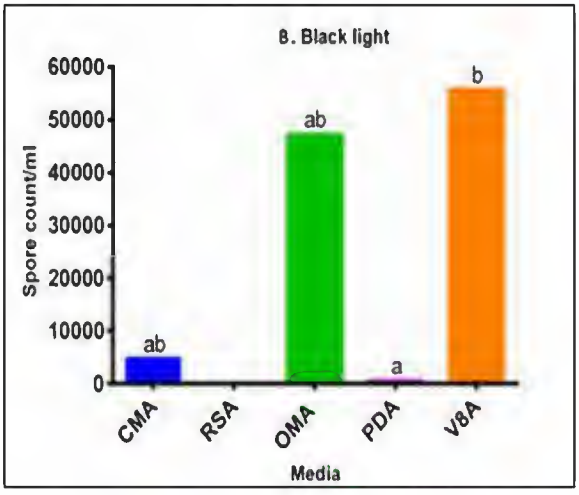


Figure 1: Black Light Wavelength Spectrum in UV Wavelength Range





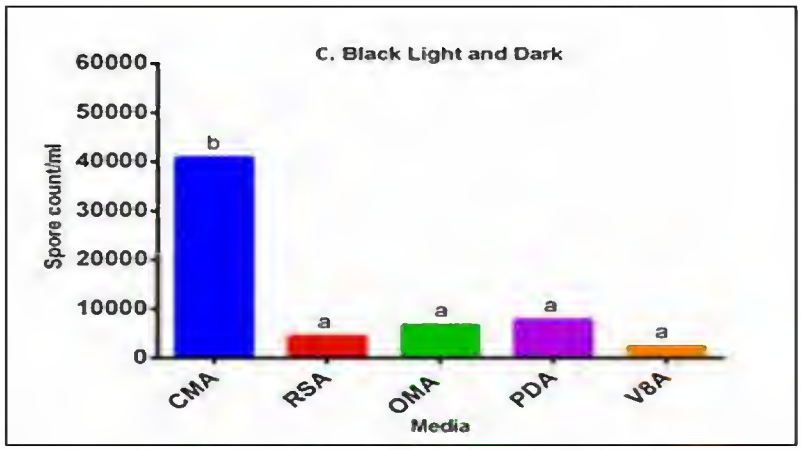


Figure 2: Sporulation of *Magnaporthe oryzae* in Different Media under A. Dark B. Black Light C. Black Light and Dark

Table 1: Mycelia Growth Measurements of 11 Day Old Culture of *Magnaporthe oryzae*

Culture Media	Mycelial Growth of *M.oryzae* on Different Media and Light Treatments in cm				
	Flourescent Light	Dark	Control	Black Light	Black Light and Dark
CMA	8.16±0.06^{ab}	8.33±0.29^{b}	7.63±0.75^{ab}	7.90±0.10^{ab}	8.46±0.46^{a}
RSA	7.53±0.46^{a}	7.16±0.06^{a}	7.33±0.29^{a}	7.33±0.31^{a}	7.60±0.35^{a}
OMA	8.73±0.31^{b}	8.40±0.53^{b}	8.83±0.29^{b}	9.00±0.00^{c}	8.66±0.29^{a}
PDA	8.60±0.72^{ab}	8.70±0.26^{b}	8.06±0.75^{ab}	8.63±0.40^{c}	8.50±0.87^{a}
V8A	9.06±0.12^{b}	8.36±0.06^{b}	8.30±0.26^{ab}	8.50±0.00^{bc}	7.96±0.55^{a}

Means in Columns Followed by the Same Letter are Not Significantly Different ($P \leq 0.05$) According to Tukey HSD

Table 2: Sporulation of *Magnaporthe oryzae* in Different Media Exposed to Different Light Conditions

Media ⇨ Light ⇩	Sporulation of *M.oryzae* on Different Media and Light Treatments				
	CMA	RSA	OMA	PDA	V8A
Fluorescent light	0 ^{a}	0 ^{a}	444 ^{a}	0 ^{a}	0 ^{a}
Dark	0 ^{a}	0 ^{a}	18333 ^{a}	10000 ^{b}	0 ^{a}
Room Condition	1333 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}
Black Light	4889 ^{a}	0 ^{a}	47111 ^{b}	444 ^{a}	55667 ^{b}
Black light (16) and Dark (8)	25556 ^{b}	1889 ^{b}	3667 ^{b}	4556 ^{ab}	778 ^{a}

Means in Columns Followed by the Same Letter are Not Significantly Different ($P \leq 0.05$) According to Tukey HSD